

What is claimed is:

- 5           1. A method for amplifying a target DNA sequence in a  
circular DNA (cDNA), comprising:
- (a) providing the cDNA template;
  - (b) adding to the cDNA template:
    - 10           (i) a first sequence-specific DNA primer that  
hybridizes to one strand of the cDNA;
    - (ii) a second sequence-specific DNA primer that is  
substantially identical to a portion of the one strand of the  
cDNA; and
    - (iii) a helicase preparation, a DNA polymerase and
    - 15           dNTPs;
  - (c) synthesizing primer extension products to  
produce:
    - (i) a plurality of copies of the target DNA sequence  
defined by the first and second primers; and
    - 20           (ii) a plurality of copies of a concatamer derived  
from the cDNA including the target; and
  - (d) amplifying the target DNA sequence.
- 25           2. A method according to claim 1, wherein the target DNA  
sequence is the entire cDNA.

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3. A method according to claim 1, wherein the target DNA sequence is a defined sequence within the cDNA bordered by the first and second primers.

5           4. A method according to claim 3, wherein the first specific sequence on the cDNA recognized by the first primer does not overlap with the second specific sequence recognized by the second primer.

10          5. A method according to claim 3, wherein the first specific sequence on the cDNA recognized by the first primer overlaps with the second specific sequence recognized by the second primer.

15          6. A method according to claim 1, wherein the cDNA is a duplex or is single-stranded or is part double-stranded with a residual part being single-stranded.

20          7. A method according to claim 1, wherein the DNA polymerase is selected from the group consisting of a T7 bacteriophage DNA polymerase, a T7-like polymerase and an exonuclease-deficient variant thereof.

25          8. A method according to claim 1, wherein the helicase preparation comprises a processive helicase.

9. A method according to claim 8, wherein the processive helicase is a hexameric-replicative helicase.

10. A method according to claim 9, wherein the processive helicase is obtained from T7 bacteriophage or T7-like bacteriophage.

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11. A method according to claim 9, wherein the helicase preparation comprises a single-strand binding protein from a T7 bacteriophage or T7-like bacteriophage.

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12. A method according to claim 1, wherein the helicase preparation comprises nucleotides.

13. A method according to claim 1, wherein the cDNA is a plasmid DNA.

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14. A method according to claim 1, wherein the cDNA has a size in the range of 50bp-500kb.

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15. A method according to claim 1, wherein the amplification in step (b) is isothermal.

16. A method according to claim 15, wherein the isothermal temperature is approximately 25°C.

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17. A method according to claim 1, wherein the cDNA is extrachromosomal DNA for detecting a pathogen.

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18. A method for amplifying a target DNA sequence in a cDNA, comprising:

(a) providing the cDNA;

5 (b) contacting the cDNA with a first sequence specific oligonucleotide primer that hybridizes to one strand of the cDNA and a second sequence specific oligonucleotide primer that is substantially identical to a portion the one strand of the cDNA, a helicase preparation, a DNA polymerase, and dNTPs;

10 (c) replicating the cDNA under conditions whereby the first primer is extended around the circle repeatedly to generate a single strand linear concatemer, and whereby the second primer hybridizes to multiple sites on a complementary single-stranded DNA which generate extension products for continuing synthesis of the target DNA sequence and the  
15 cDNA; and

(d) amplifying the target cDNA.

20 19. A method for amplifying a target DNA sequence in a cDNA, comprising:

(a) annealing a first sequence specific oligonucleotide primer to a first DNA sequence adjacent to or within a target DNA sequence in a cDNA in the presence of a polymerase and a helicase preparation to synthesize by primer extension, a  
25 displaced single stranded DNA containing a plurality of copies of the target DNA sequence;

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(b) annealing a second specific oligonucleotide primer to the displaced strand of DNA at specific sites for synthesizing by primer extension, a plurality of complementary single stranded DNA concatamers, each concatamer including the target DNA sequence and each concatamer forming a substrate for multiple rounds of displacement synthesis from the first or second primer; and

(c) amplifying the target DNA sequence.

10        20. An amplification kit comprising: a helicase preparation containing one or more helicases, a DNA polymerase and instructions for performing helicase-dependent amplification of circular nucleic acids according to claim 1.

15        21. An amplification kit according to claim 20, wherein the helicase preparation comprises: T7 gene 4B helicase, T7 gene 2.5 SSB protein, dTTP or ATP, T7 Sequenase.

20        22. An amplification kit according to claim 21, wherein the helicase preparation further comprising one or more cofactors including an accessory protein, a set of four deoxynucleotides and optionally a reaction buffer.

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